

## BRIEF REPORT

# Studies on antitumor activity spectrum of doxycycline

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## ABSTRACT

In this study, *in vitro* (21 kinds of cell lines) and *in vivo* (four kinds of tumor-bearing mouse models) experiments were performed to determine the anticancer effect of doxycycline. This drug may elicit a strong inhibitory effect on cancer cells and improve the survival condition of mice. This study also preliminarily investigated the inhibitory effect of doxycycline on different kinds of tumor cells.

**Key Words:** Doxycycline, Cancer cell, Antitumor, Cell proliferation

## 1. INTRODUCTION

Novel efficacy of “old” drugs have been extensively investigated for use as new treatments. For instance, antibiotics can be used as anticancer drugs.<sup>[1-4]</sup> Four to five classes of drugs, such as tetracyclines, can also be used to eradicate 12 cancer cell lines.<sup>[5]</sup> Tetracyclines are cytotoxic to tumor cells.<sup>[6]</sup> As a semi-synthetic tetracycline, doxycycline inhibits matrix metalloproteinase (MMP) activation, prevents cell proliferation, and downregulates DNA-dependent protein kinase.<sup>[7,8]</sup>

## 2. MATERIALS AND METHODS

### 2.1 Materials

Doxycycline was purchased from Sangon Biotech (Shanghai, China). Cyclophosphamide was procured from Alfa-Aesa (Ward Hill, USA). All of the cell lines were supplied by Kaiji Biotech (Nanjing, China). Cell culture media were obtained from Hyclone (Waltham, USA). Mice were purchased from the Academy of Military Medical Sciences of the Chinese PLA (Beijing, China).

### 2.2 Methods

All of the cell lines were cultured in a medium supplemented with 10% (v/v) heat-inactivated (56°C, 30 min) fetal calf serum (Hyclone, USA) and maintained at 37°C in humidified air containing 5% CO<sub>2</sub>. PC-3 cells were cultured in F12K medium. NCI-H446, A549, PLC/PRF/5, SMMC-7721, MuM2B, MuM2C, MCF-7, SGC-7901, SH-SY5Y, K562, and HL60 cells were cultured in RPMI1640 medium. HepG-2, MHCC97H, MHCC97L, LOVO, A875, A375, MDA-MB-231, PANC-1, ASPC-1, and HeLa cells were cultured in DMEM (high glucose) medium.

Cell viability was determined via an MTT assay. The cells ( $5 \times 10^3$  cells/well) were seeded in 96-well culture plates. The cells were incubated overnight. Afterward, they were treated with various doxycycline concentrations (0.00, 0.39, 0.78, 1.56, 3.12, 6.25, 12.5, 25.00, 50.00, and 100.00  $\mu$ M). After 48 h of incubation, 20  $\mu$ l of MTT was added at 37°C for 4 h, and cell viability was determined. Then, 150  $\mu$ l of dimethyl sulfoxide was added to dissolve the formazan crystals. Opti-

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cal density was determined at 570 nm by using a microplate reader (Multiskan™ FC, Thermo Scientific, Waltham, MA, USA).

Five- to six-week-old male C57BL/6 and BA1B/c nu/nu mice were prepared for mouse and human tumorigenesis, respectively. They were maintained in a specific pathogen-free animal care facility in accordance with institutional guidelines. Tumor xenografts were established by subcutaneously injecting  $1 \times 10^7$  cells suspended in phosphate buffered saline into the flank. The mice were randomly divided into five groups (n = 10/group) 1 day after the tumor cells were inoculated. After the tumors reached an approximate volume of 100 mm<sup>3</sup> (approximately 6 weeks after injection), the mice were treated with 60, 30, or 15 mg/kg doxycycline, 20 mg/kg cyclophosphamide, and saline via oral gavage once a day. Their body weights were measured at different time points after the tumor cells were inoculated. Tumor diameters were also measured every day. Tumor volumes were calculated according to the following equation:  $V = ab^2/2$ , where a is the tumor length and b is the tumor width. Seven weeks after the treatment was administered, all of the mice were euthanized, and xenografts were resected and measured. The inhibitory rates were calculated as  $(\%) = (1 - T_i/C_i) \times 100\%$ , where  $T_i$  is the tumor volume of the treated group and  $C_i$  is the tumor volume of the control group. Changes in weight were determined as  $(\%) = (T_w/C_w - 1) \times 100\%$ , where  $T_w$  is the body weight of the treated group and  $C_w$  is the body weight of the control group.

Data were expressed as means  $\pm$  standard deviation. Comparisons between the groups were performed through one-way ANOVA, followed by Bonferroni post hoc test (SPSS version 17.0, SPSS Inc., Chicago, IL, USA). The significance level was set at  $P < .05$ .

### 3. RESULTS

Performing the MTT assay, we determined the effect of the 48 h doxycycline treatment on the cell viability of various cancer cell lines. We measured IC<sub>50</sub> of the different cell lines to doxycycline. Doxycycline significantly inhibited the proliferation of these cells in a dose-dependent manner (see Table 1). IC<sub>50</sub> of most cells was less than 5  $\mu$ M, which was quite low. This result demonstrated that many cancer cell lines were very sensitive to doxycycline.

Tumor formation ability is an important indicator of cancer cell malignancy. Therefore, we investigated the inhibitory effect of doxycycline on tumor growth *in vivo* by using four tumor-bearing mouse xenograft models.

**Table 1.** IC<sub>50</sub> dose of doxycycline for different cancer cell lines

Cells	IC <sub>50</sub> ( $\mu$ M)	
Melanoma cells	A875	3.10 $\pm$ 0.33
	A375	2.42 $\pm$ 0.14
	Mum2B	2.75 $\pm$ 0.55
	Mum2C	1.40 $\pm$ 0.11
Lung cancer cells	NCI-H446	1.70 $\pm$ 0.12
	A549	1.06 $\pm$ 0.13
Hepatoma carcinoma cell	PLC	2.23 $\pm$ 0.15
	MHCC97H	10.05 $\pm$ 3.94
Breast cancer cells	MCF-7	2.09 $\pm$ 0.03
	MDA-MB-231	3.61 $\pm$ 0.37
Leukemia cells	K562	1.45 $\pm$ 0.10
	HL60	1.48 $\pm$ 0.22
Pancreatic cancer	PCNA-1	34.54 $\pm$ 6.79
	AsPC-1	29.34 $\pm$ 6.17
Human embryonic kidney cells	HEK 293	11.85 $\pm$ 1.07
	HEK 293T	2.47 $\pm$ 0.39
Gastric cancer cells	SGC-7901	2.45 $\pm$ 0.26
Cervical cancer cells	Hela	4.08 $\pm$ 0.19
Neuroblastoma	SH-SY5Y	3.70 $\pm$ 0.14
Colon cancer	LOVO	0.91 $\pm$ 0.15
Prostate cancer cells	PC-3	2.35 $\pm$ 0.13

Note. Each experiment was performed in triplicate. Results show the means of the three experiments, and the error bars represent standard deviation.

We analyzed the changes in the tumor volume and body weights of the mice treated with doxycycline and then compared them with those observed in the control group. The inhibition effect of doxycycline on the tumor cells was very evident compared with that of the positive drug control group (see Table 2). Doxycycline could significantly inhibit the proliferation of B16 melanoma cells, Lewis lung cancer cells, MCF-7 breast cancer cells, and NCI-H446 human small cell lung cancer cells.

The body weight of the doxycycline-treated mice was significantly higher than that of the control group. The body weights of the mice in the low-, middle-, and high-dose groups respectively treated with 15, 30, and 60 mg/kg doxycycline were also significantly higher than those of the mice

in the cyclophosphamide-treated group.

Doxycycline increased the body weight of the mice and improved their survival conditions. This finding indicated that doxycycline exhibited a very good antitumor activity and

caused few side effects. Doxycycline also elicited remarkable anti-tumor effects. Therefore, this drug can be applied to treat and prevent cancer.

**Table 2.** The changes of tumor volume and body weight compared with control group

Target drug	Observation							
	Inhibiting rates (%)				Weight change (%)			
	B16	Lewis	NCI-H446	MCF-7	B16	Lewis	NCI-H446	MCF-7
Cyclophosphamide (20 mg/kg)	60.7	47.3	87.5	63.4	-14.3	-7.1	-14.0	-21.9
15 mg/kg	45.2	64.9	33.1	27.5	+8.2	+4.1	-2.4	-2.3
Doxycycline 30 mg/kg	56.3	74.2	57.1	41.2	+13.3	+7.4	-2.7	+2.3
60 mg/kg	80.4	81.2	67.9	96.0	+17.7	+9.6	-7.8	+6.8

#### 4. DISCUSSION

Doxycycline influences cell adhesion processes and the activity of focal adhesion kinase; for instance, this drug prevents cell adhesion during migration.<sup>[9]</sup> Doxycycline induces the membrane expression of VE-cadherin on endothelial cells and prevents vascular hyperpermeability.<sup>[10]</sup> Besides, doxycycline also inhibits metabolism and metastasis by interfering with MMPs and E-cadherin levels.<sup>[11]</sup> Previous study had proved decreasing MMP activity could inhibit cancer cell invasion and metastasis, decreasing of E-cadherin expression is sufficient to confer metastatic ability to breast cancer.<sup>[12]</sup> Doxycycline increases E-cadherin levels, decreases vimentin protein expression, which are two markers of invasiveness and metastasis, and inhibits EMT-related transcription fac-

tor activities.<sup>[13,14]</sup> Therefore, this drug elicits an antitumor effect through the combined action of various targets.

Our study revealed that doxycycline elicits a remarkable inhibitory effect on cancer cells. In our animal experiments, the inhibitory rates of doxycycline were higher than those of cyclophosphamide. Doxycycline could also improve the survival condition of mice. Therefore, doxycycline is a promising anticancer agent because it inhibits cancer cell proliferation, induces less toxic effects and causes fewer side effects than other drugs do.

#### CONFLICTS OF INTEREST DISCLOSURE

The author declares that there is no conflict of interest statement.

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